Amendments to the Specification

Please amend the paragraph appearing at page 1, lines 14-22, of the specification as follows:

~~INCORPORATION BY REFERENCE~~

~~All references referred to herein are hereby incorporated by reference in their entirety. The incorporation of these references, standing alone, should not be construed as an assertion or admission by the inventors that any portion of the contents of all of these references, or any particular reference, is considered to be essential material for satisfying any national or regional statutory disclosure requirement for patent applications. Notwithstanding, the inventors reserve the right to rely upon any of such references, where appropriate, for providing material deemed essential to the claimed invention by an examining authority or court. No reference referred to herein is admitted to be prior art to the claimed invention.~~

Please amend the specification at page 4, lines 14-25, as follows:

In addition to anionic groups, polynucleotide probes featured in the present invention may further include cationic and/or nonionic groups, provided the probes have a net ~~positive~~ negative charge. The polynucleotide may consist of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), a combination of DNA and RNA, or it may include a nucleic acid analog (*e.g.*, a peptide nucleic acid) or contain one or more modified nucleosides (*e.g.*, a ribonucleoside having a 2'-O-methyl substitution to the ribofuranosyl moiety). Non-nucleotide groups, such as polysaccharides or polyethyelene glycol, may also be included in the probes, provided they do not prevent or substantially interfere with hybridization of the probe to the target nucleic acid . Probes of the present invention are up to 100 bases or more in length (preferably from 12 to 50 bases, and more preferably from 18 to 35 bases in length) and contain a base region which is complementary to a target sequence contained in the target nucleic acid (the base region is preferably perfectly complementary to the target sequence).

Please amend the paragraph bridging pages 5 and 6 of the specification as follows:

The target nucleic acid may be RNA or DNA, a nucleic acid analog or a chimeric containing different types of nucleic acid and/or nucleic acid analogs. A preferred target nucleic acid of the present invention is RNA, especially ribosomal RNA (rRNA) and messenger RNA (mRNA). Ribosomal RNA is a preferred target nucleic acid for detecting groups of organisms in test samples because of its relative abundance in cells and because of its conserved nature which allows for differentiating between defined groups of organisms. *See, e.g.*, Kohne, "Method for Detecting, Identifying, and Quantitating Organisms and Viruses," U.S. Patent No. 5,288,611, and Hogan *et al.*, "Nucleic Acid Probes for Detection and/or Quantitation of Non-Viral Organisms," U.S. Patent No. 5,840,488, the contents of each of which is hereby incorporated by reference herein. For measuring gene expression, determining the presence of a particular cell-type, or detecting the presence of a target group of viruses, assaying for specific mRNAs may be preferred. *See, e.g.*, Gentalen *et al.*, "Methods of Using an Array of Pooled Probes in Genetic Analysis," U.S. Patent No. 6,306,643; Kohne, "Method for Detecting the Presence of RNA Belonging to an Organ or Tissue Cell-Type," U.S. Patent No. 5,932,416; and Kohne, "Method for Detecting the Presence of Group-Specific Viral mRNA in a Sample," U.S. Patent No. 5,955,261, the contents of each of which is hereby incorporated by reference herein.

Please amend the paragraph bridging pages 7 and 8 of the specification as follows:

In a further embodiment of the present invention, a kit is featured which comprises: (i) a polynucleotide probe which preferentially hybridizes to a target nucleic acid sequence in a test sample under hybridization assay conditions; and (ii) a synthetic polycationic polymer in an amount sufficient to increase the association rate of the probe and the target sequence in the test sample under the hybridization assay conditions. The probe and polymer may be provided in the same or separate containers. Kits according to the present invention may further comprise at least one of the

following: (i) a reagent to dissociate the polymer from the probe; (ii) one or more amplification primers for amplifying a target sequence contained in or derived from the target nucleic acid; (iii) a capture probe for isolating and purifying target nucleic acid present in a test sample; and (iv) if a capture probe is included, a solid support material (*e.g.*, magnetically responsive particles) for immobilizing the capture probe, either directly or indirectly, in a test sample. Where the target nucleic acid is a structured nucleic acid having regions of self-complementarity, such as rRNA, the kits of the present invention may further include helper probes. *See* Hogan *et al.*, "Means and Method for Enhancing Nucleic Acid Hybridization," U.S. Patent No. 5,030,557, the contents of which are hereby incorporated by reference herein. Additionally, the kits may comprise written instructions for performing an assay to determine the presence or absence of a target nucleic acid sequence in the test sample as an indication of the presence or absence of a target virus or organism or members of a target group of viruses or organisms in the test sample. The assay described in the written instructions may include steps for isolating and purifying the target nucleic acid prior to detection with the polynucleotide probe and/or amplifying a target sequence contained in the target nucleic acid. Alternatively, the kit may include written instructions for performing, for example, an assay to detect the presence of a disease-associated gene, to determine the state of a disease, to measure levels of gene expression, or to detect mutations or polymorphisms in a test sample.

Please amend the paragraph bridging pages 10 and 11 of the specification as follows:

By "polynucleotide" is meant a polymer having two or more nucleoside subunits or nucleobase subunits coupled together. The polynucleotides include DNA and/or RNA or analogs thereof and may further include non-nucleotide groups such as, for example, abasic nucleotides, universal bases (*e.g.*, 3-nitropyrrole and 5-nitroindole), polysaccharides, peptides, polypeptides and/or polyethylene glycol. *See, e.g.*, Becker *et al.*, "Molecular Torches," U.S. Patent No. 6,361,945; ~~U.S. application Serial No. 09/346,551 and International Publication No. WO 00/01850, both of which enjoy common ownership herewith;~~ Bergstrom *et al.*, "3-Nitropyrrole Nucleoside," U.S.

Patent No. 5,681,947; Loakes *et al. Nucleic Acids Research* (1995) 23(13):2361-2366; and Arnold *et al.*, "Linking Reagents for Nucleotide Probes," U.S. Patent No. 5,585,481. The sugar groups of the nucleoside subunits may be ribose, deoxyribose and analogs thereof, including, for example, ribonucleosides having a 2'-O-methyl substitution to the ribofuranosyl moiety. (Polynucleotides including nucleoside subunits having 2' substitutions which are useful as polynucleotide probes are disclosed by Becker *et al.*, "Method for Amplifying Target Nucleic Acid Using Modified Primers," U.S. Patent No. 6,130,038.) The nucleoside subunits may by joined by linkages such as phosphodiester linkages, modified linkages or by non-nucleotide moieties which do not prevent hybridization of the polynucleotide to its complementary target nucleic acid sequence. Modified linkages include those linkages in which a standard phosphodiester linkage is replaced with a different linkage, such as a phosphorothioate linkage or a methylphosphonate linkage. The nucleobase subunits may be joined, for example, by replacing at least a portion of the natural deoxyribose phosphate backbone of DNA with a pseudo peptide backbone, such as a 2-aminoethylglycine backbone which couples the nucleobase subunits by means of a carboxymethyl linker to the central secondary amine. (DNA analogs having a pseudo peptide backbone are commonly referred to as "peptide nucleic acids" or "PNA" and are disclosed by Nielsen *et al.* in U.S. Patent No. 5,773,571.) Other non-limiting examples of polynucleotides contemplated by the present invention include nucleic acid analogs containing bicyclic and tricyclic nucleoside and nucleotide analogs referred to as "Locked Nucleic Acids," "Locked Nucleoside Analogues" or "LNA." (Locked Nucleic Acids are disclosed by Wang, "Conformationally Locked Nucleosides and Oligonucleotides," U.S. Patent No. 6,083,482; Imanishi *et al.* in U.S. Patent No. 6,268,490; and Wengel *et al.*, "Oligonucleotide Analogues," ~~International Publication No. WO 99/14226~~ U.S. Patent No. 6,670,461.) Any nucleic acid analog is contemplated by the present invention provided the modified polynucleotide can form a stable hybrid with a target nucleic acid under hybridization assay conditions and at least a portion of the modified polynucleotide is anionic. In the case of polynucleotide probes, the modified polynucleotide must be capable of preferentially hybridizing to the target nucleic acid under hybridization assay conditions. Unless indicated to be a "probe," a

polynucleotide, as used herein, may be a nucleic acid molecule obtained from a natural source which is at least partially single-stranded or which may be rendered partially or fully single-stranded by human intervention.

Please amend the paragraph bridging pages 11 and 12 of the specification as follows:

By "polynucleotide probe" or "probe" is meant a polynucleotide having a base sequence sufficiently complementary to its target nucleic acid sequence to form a probe:target hybrid stable for detection under hybridization assay conditions. As would be understood by someone having ordinary skill in the art, a probe is an isolated nucleic acid molecule, or an analog thereof, in a form not found in nature without human intervention (*e.g.*, recombined with foreign nucleic acid, isolated, or purified to some extent). Probes may have additional nucleosides or nucleobases outside of the targeted region so long as such nucleosides or nucleobases do not prevent hybridization under hybridization assay conditions and, where indicated, do not prevent preferential hybridization to the target nucleic acid. A non-complementary sequence may also be included, such as a target capture sequence (generally a homopolymer tract, such as a poly-A, poly-T or poly-U tail) or sequences which will confer a desired secondary or tertiary structure, such as a hairpin structure, which can be used to facilitate detection. Examples of probes having a target capture sequence ("capture probes") are disclosed by Ranki *et al.*, "Detection of Microbial Nucleic Acids by a One-Step Sandwich Hybridization Test," U.S. Patent No. 4,486,539; Stabinsky, "Methods and Kits for Performing Nucleic Acid Hybridization Assays," U.S. Patent No. 4,751,177; and Weisburg *et al.*, "Two Step Hybridization and Capture of a Polynucleotide," U.S. Patent No. 6,110,678, the contents of each of which is hereby incorporated by reference herein. Self-hybridizing probes are disclosed by, for example, Bagwell, "Fluorescent Imperfect Nucleic Acid Probes," U.S. Patent No. 5,607,834; Tyagi *et al.*, "Detectably Labeled Dual Conformation Oligonucleotide Probes, Assays and Kits," U.S. Patent No. 5,925,517; and Becker *et al.* in U.S. Patent No. 6,361,945, the contents of each of which

is hereby incorporated by reference herein. ~~in U.S. application Serial No. 09/346,551 and International Publication No. WO 00/01850.~~

Please amend the specification at page 16, lines 19-30, as follows:

By "label" is meant a reporter moiety associated with a polynucleotide which can be detected by means well known in the art and used to indicate the presence or absence of a particular polynucleotide sequence in a test sample. Examples of labels which are well known in the art include chemiluminescent, electrochemiluminescent and fluorescent compounds, radioisotopes, dyes, polynucleotides, enzymes, enzyme substrates, chromophores and haptens. When multiple interacting labels are associated with a polynucleotide, interacting labels may include, for example, the following: luminescent and quencher labels, luminescent and adduct labels, dye dimer labels, enzyme and substrate labels, enzyme and cofactor labels, and Förrester energy transfer pairs. Examples of polynucleotides having multiple interacting labels are disclosed by, for example, Bagwell in U.S. Patent No. 5,607,834; Tyagi *et al.* in U.S. Patent No. 5,925,517; and Becker *et al.* in U.S. Patent No. 6,361,945. ~~U.S. application Serial No. 09/346,551 and International Publication No. WO 00/01850.~~

Please amend the paragraph bridging pages 20 and 21 of the specification as follows:

Merely identifying a putatively unique potential target nucleotide sequence does not guarantee that a functionally specific polynucleotide probe may be made to hybridize to nucleic acid comprising that sequence. Various other factors will determine the suitability of a nucleic acid locus as a target site for a specific probe. Because the extent and specificity of hybridization reactions, such as those described herein, are affected by a number of factors, manipulation of one or more of those factors will determine the exact sensitivity and specificity of a particular polynucleotide, whether perfectly complementary to its target or not. The importance and effect of various hybridization assay conditions are known to those skilled in the art and are disclosed by, for example, Kohne, "Method

for Detection, Identification and Quantitation of Non-Viral Organisms," U.S Patent No. 4,851,330; Hogan *et al.*, "Nucleic Acid Probes to *Mycobacterium gordonae*," U.S. Patent No. 5,216,143; and Hogan, "Nucleic Acid Probes for Detection and/or Quantitation of Non-Viral Organisms," U.S. Patent Nos. 5,840,488, the contents of each of which is hereby incorporated by reference herein.

Please amend the paragraph bridging pages 21 and 22 of the specification as follows:

While probes having extensive self-complementarity are generally avoided, there are some applications in which probes exhibiting at least some degree of self-complementarity are desirable to facilitate detection of probe:target duplexes in the presence of unhybridized probe. By way of example, structures referred to as "Molecular Torches" are designed to include distinct regions of self-complementarity (coined the "target binding domain" and the "target closing domain") which are connected by a polynucleotide and/or non-nucleotide joining region and hybridize to one another under predetermined hybridization assay conditions. When exposed to denaturing conditions, the two complementary regions (which may be fully or partially complementary) of the Molecular Torch melt, leaving the target binding domain available for hybridization to a target sequence when the predetermined hybridization assay conditions are restored. Molecular Torches are designed so that the target binding domain favors hybridization to the target sequence over the target closing domain. The target binding domain and the target closing domain of a Molecular Torch include interacting labels (*e.g.*, luminescent/quencher) positioned so that a different signal is produced when the Molecular Torch is self-hybridized than when the Molecular Torch is hybridized to a target nucleic acid, thereby permitting detection of probe:target duplexes in a test sample in the presence of unhybridized probe having viable labels associated therewith. (Molecular Torches are disclosed by Becker *et al.* in U.S. Patent No. 6,361,945, ~~U.S. application Serial No. 09/346,551 and International Publication No. WO 00/01850.~~) In accordance with the teachings of Becker, probes of the present invention may be designed and constructed to include, in addition to a "target binding domain" able to distinguish target nucleic acid from non-

target nucleic acid, a "target closing domain," a "joining region" and interacting labels characteristic of a Molecular Torch.

Please amend the paragraph bridging pages 23 and 24 of the specification as follows:

A preferred method for determining T_m measures hybridization using the Hybridization Protection Assay (HPA) disclosed by Arnold *et al.*, "Homogenous Protection Assay," U.S. Patent No. 5,283,174, the contents of which are hereby incorporated by reference herein. The T_m can be measured using HPA in the following manner. Probe molecules are labeled with an acridinium ester and permitted to form probe:target hybrids in a lithium succinate buffer (0.1 M lithium succinate buffer, pH 4.7, 20 mM EDTA, 15 mM aldrithiol-2, 1.2 M LiCl, 3% (v/v) ethanol absolute, 2% (w/v) lithium lauryl sulfate) using an excess amount of target. Aliquots of the solution containing the probe:target hybrids are then diluted in the lithium succinate buffered solution and incubated for five minutes at various temperatures starting below that of the anticipated T_m (typically 55°C) and increasing in 2-5°C increments. This solution is then diluted with a mild alkaline borate buffer (600 mM boric acid, 240 mM NaOH, 1% (v/v) TRITON® X-100 (octoxynol), pH 8.5) and incubated at an equal or lower temperature (for example 50°C) for ten minutes.

Please amend the specification at page 27, lines 11-22 as follows:

Non-isotopic materials can also be used for labeling and may be introduced internally into the nucleic acid sequence or at the end of the nucleic acid sequence. Modified nucleotides may be incorporated enzymatically or chemically. Chemical modifications of the probe may be performed during or after synthesis of the probe, for example, through the use of non-nucleotide linker groups, as disclosed by Arnold *et al.* in U.S. Patent No. 6,031,091. Non-isotopic labels include fluorescent molecules (individual labels or combinations of interacting labels, such as the fluorescence resonance energy transfer (FRET) pairs disclosed by Tyagi *et al.* in U.S. Patent No. 5,925,517), chemiluminescent molecules, enzymes, cofactors, enzyme substrates, haptens or other ligands. The

probes of the present invention are preferably labeled by means of a non-nucleotide linker with an acridinium ester (AE). Acridinium ester labeling may be performed as disclosed by Arnold *et al.*, "Acridinium Ester Labelling and Purification of Nucleotide Probes," U.S. Patent No. 5,185,439, the contents of which are hereby incorporated by reference herein.

Please amend the specification at page 35, lines 9-23, as follows:

An extraction method requiring only a single reagent to release nucleic acids from a wide range of cellular types in a form suitable for nucleic acid hybridization without the need for subsequent purification steps is disclosed by Clark *et al.*, "Method for Extracting Nucleic Acids from a Wide Range of Organisms," U.S. Patent No. 5,786,208, the contents of which are hereby incorporated by reference herein. This extraction method combines a test sample with a reagent which includes a non-ionic detergent, an optional anionic detergent and a metal chelating agent and heats the resulting mixture at a temperature between 80° and 100°C until nucleic acids are released from the cells. Because anionic detergents such as lithium lauryl sulfate are believed to disrupt or denature nucleases (*e.g.*, ribonucleases) present in the test sample, inclusion of an anionic detergent is particularly desirable when the target nucleic acid is RNA. (In this and other extraction methods employing an anionic detergent, the target nucleic acid is preferably separated from the anionic detergent prior to contacting the target nucleic acid with the polycationic polymers of the present invention.) Nucleic acids are released in this method without observable destruction to cell walls, so that the liberated nucleic acids are suitable for hybridization, amplification or other genetic manipulations without further purification.

Please amend the paragraph bridging pages 35 and 36 of the specification as follows:

Following sample preparation, the polycationic polymers of the present invention may be used in a variety of detection systems, including both heterogenous and homogenous systems used to determine the presence or amount of target nucleic acids in a sample. In a heterogenous assay,

a step is required to isolate or separate probe:target hybrids from excess probe sequences before single-stranded probes and probe:target hybrids can be distinguished from each other. Examples of heterogenous assay systems are disclosed by, for example, Ranki *et al.* in U.S. Patent No. 4,486,539 and Stabinsky in U.S. Patent No. 4,751,177. Homogenous assays on the other hand require no separation step, thereby permitting the in solution detection of probe:target hybrids in the presence of excess probe sequences. Examples of detection systems which can be used in either the heterogenous or homogenous systems are the Hybridization Protection Assay and the Adduct Protection Assay disclosed by Arnold *et al.* in U.S. Patent No. 5,283,174, and Becker *et al.*, "Adduct Protection Assay," U.S. Patent No. 5,731,148 (the contents of which are hereby incorporated by reference herein), respectively. Other well known detection systems employ self-hybridizing probes which incorporate interacting labels that emit differentially detectable signals, depending upon whether the probes are bound to target nucleic acid or remain self-hybridized in the reaction mixture. *See, e.g.*, Bagwell in U.S. Patent No. 5,607,834; Tyagi *et al.* in U.S. Patent No. 5,925,517; and Becker *et al.* in U.S. Patent No. 6,361,945. ~~U.S. application Serial No. 09/346,551 and International Publication No. WO 00/01850~~.

Please amend the paragraph bridging pages 37 and 38 of the specification as follows:

Prior to detection, it may be desirable to increase the quantity of target nucleic acid present, and thus the sensitivity of the assay, by exposing the reaction mixture to nucleic acid amplification conditions. Under amplification conditions, polynucleotide chains containing the target sequence or its complement are synthesized in a template-dependent manner from ribonucleoside or deoxynucleoside triphosphates using nucleotidyltransferases known as polymerases. There are many amplification procedures in common use today, including the polymerase chain reaction (PCR), Q-beta replicase, self-sustained sequence replication (3SR), transcription-mediated amplification (TMA), nucleic acid sequence-based amplification (NASBA), ligase chain reaction (LCR), strand displacement amplification (SDA) and loop-mediated isothermal

amplification (LAMP), each of which is well known in the art. *See, e.g.*, Mullis, "Process for Amplifying Nucleic Acid Sequences," U.S. Patent No. 4,683,202; Erlich *et al.*, "Kits for Amplifying and Detecting Nucleic Acid Sequences," U.S. Patent No. 6,197,563; Walker *et al. Nucleic Acids Res.* (1992) 20:1691-1696; Fahy *et al. PCR Methods and Applications* (1991) 1:25-33; Kacian *et al.*, U.S. Patent No. 5,399,491; Kacian *et al.*, "Nucleic Acid Sequence Amplification Methods," U.S. Patent No. 5,480,784; Davey *et al.*, "Nucleic Acid Amplification Process," U.S. Patent No. 5,554,517; Birkenmeyer *et al.*, "Amplification of Target Nucleic Acids Using Gap Filling Ligase Chain Reaction," U.S. Patent No. 5,427,930; Marshall *et al.*, "Amplification of RNA Sequences Using the Ligase Chain Reaction," U.S. Patent No. 5,686,272; Walker, "Strand Displacement Amplification," U.S. Patent No. 5,712,124; Notomi *et al.*, "Process for Synthesizing Nucleic Acid," European Patent Application No. 1 020 534 A1; Dattagupta *et al.*, "Isothermal Strand Displacement Amplification," U.S. Patent No. 6,214,587; and HELEN H. LEE ET AL., NUCLEIC ACID AMPLIFICATION TECHNOLOGIES: APPLICATION TO DISEASE DIAGNOSIS (1997). The contents of each of the foregoing references is hereby incorporated by reference herein.

Please amend the paragraph appearing at page 40, lines 4-18, of the specification as follows:

Instrument systems for performing detection assays are well known in the art and may be used to perform manual, semi-automated or fully automated assays. Some of these instrument systems are limited to direct detection (no prior amplification step), while others have the capability of performing both amplification and detection. These instrument systems may detect the formation of polynucleotide hybrids using any of a variety of techniques known in the art including, but not limited to, those based on light emission, mass changes, changes in conductivity or turbidity. Examples of instrument systems which could be readily adapted to perform assays incorporating the polycationic polymers of the present invention in order to enhance reaction rates include those sold under the trade names of DTS 400 (detection only) and DTS 1600 (amplification and detection) by Gen-Probe Incorporated of San Diego, CA, which represent embodiments of instrument systems

disclosed by Acosta *et al.*, "Assay Work Station," U.S. Patent No. 6,254,826, and by Ammann *et al.*, "Automated Process for Isolating and Amplifying a Target Nucleic Acid Sequence," U.S. Patent No. 6,335,166. ~~U.S. application Serial No. 09/303,030, and International Publication No. WO 99/57561, "Automated Diagnostic Analyzer and Method," each of which enjoys common ownership herewith.~~